



Assessment of the content of heavy metals and potential pathogenic microorganisms in soil under illegal dumping sites

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Abstract The aim of the study was the assessment of the environmental contamination of soil by heavy metals and microorganisms within the uncontrolled landfill dumps and the impact of anthropogenic sources on the activity of selected soil surface horizons. Some physicochemical properties were determined according to commonly used procedures in soil science laboratories. The total of heavy metals concentration was assessed according to Crock and Severson and forms available according to Lindsay and Norvell. The total contents and mobile forms were determined by atomic absorption spectrophotometry. As part of the microbiological tests, potentially pathogenic evidence of bacteria was found indicating fecal contamination of the soil (*E. coli* and other coliform bacilli, *Salmonella* spp., and *Enterococcus* spp.). Quantitative analyses were carried out based on the calculation of the most probable number of microorganisms (MPN method). The activity of selected redox and hydrolases enzymes: the activity of dehydrogenases with the Thalmann method, catalase with the Johnson and Temple method, alkaline and acid phosphatase with the method of Tabatabai and Bremner. It was concluded that

depending on the composition of the dump, the physicochemical properties of the soil were changed, too. Uncontrolled landfills significantly affected the increase or decrease in the pH of the tested soil and the organic content of the tested soil. The waste stored at the site affected the contamination of the soil by heavy metals to varying degrees. The results show that the most dangerous bacteria exist in the soil under the C landfill and this is where the greatest risk of soil contamination exists. The highest amount of selected enzymes activity was observed in soils under illegal C dumping sites. In the soil sourced from A and B landfills, the enzyme inhibition occurred which was linked to the lowering of the organic carbon. The results of the principal component analysis demonstrated that the content of heavy metals and the activity of enzymes are the indicators of anthropogenic pollution, whereas granulometric composition (sand and silt) is mainly associated with the natural environment.

Keywords Illegal waste storage · Heavy metals · Microorganisms · Soil enzymes

Introduction

With the increasing standard of living, the prosperity and the development of technology, a substantial amount of waste is being produced. To deal with the waste deposit, landfills have been created. They are well planned, managed, designed, and located in terms of technical requirements. Despite the advancement in the environmental protection and ecology, the state of waste management is still unsatisfactory (Al-Khatib et al. 2015). Waste is often deposited by the local community in random places in unlisted (random) dumps. As a consequence, such illegal landfills are a potential risk of contamination source since

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they are not secured in any way. As a result, such landfills cause a transfer of toxic substances into the soil (Kaszubkiewicz et al. 2011; Talala 2014). Open dumpsites present a number of risks to soils due to its propensity to generate toxic chemicals, pathogens, and alter the natural environment of the soil (Amuno 2011). The source of heavy metals is usually hazardous waste (paint, varnishes, batteries, expired pharmaceuticals, insecticides), ashes coming from the heating systems as well as the organic fraction which has a substantial accumulation ability (Longe and Enekwechi 2007; Tengrui et al. 2007; Ogundiran and Afolabi 2008; Islam et al. 2012). Uncontrolled landfills are also an important epidemiological risk due to the possibility of pathogenic bacteria being deposited waste. In particular, the waste containing food waste, including cooking food waste, food packaging, used hygiene materials, as well as pet feces are sources of particular microbiological spectrum. Their count is 10^8 – 10^9 per gram of waste. Bacteria such as typhoid bacillus (*Salmonella* spp.), fecal bacteria (*Escherichia coli* and other coliform bacilli) and fecal streptococci (*Enterococcus* spp.) are a particular threat to the environment and human health (Kalwasinska and Burkowska 2013; Yamahara et al. 2012).

The soil in and around landfills may become inhibited permanently or temporarily by pathogenic microorganisms. The microorganisms can survive and exists in the ground water and crops in the vicinity of landfills. The pathogenic bacteria can survive in the soil anytime between a couple of hours to several months. The reduction in their number to a value not endangering the health of humans occurs within two to three months. The rate of reduction of fecal bacteria in the soil depends on the temperature, pH, soil moisture, the season, and the presence of the antagonistic microflora in the soil against the pathogens (Grisey et al. 2010). Bacteria of the genus *Salmonella*, coliform bacilli including *E. coli* and enterococci are the indicators of the sanitary condition of the soil, because their presence might endanger the health of humans and animals (Edrington et al. 2009). They usually cause food poisoning in people and according to the Polish norms they should not exist in the soil. The detailed knowledge of the impact of uncontrolled landfills on the soil environment is an important and challenging issue requiring a comprehensive physicochemical and microbiological research, because these landfills are often poorly monitored.

The activity in soil enzymes is very important as an indicator for soil biological quality (Rejsek et al. 2012; Bartkowiak and Lemanowicz 2014; Lemanowicz and Krzyżaniak 2015). These activities are informative to determine changes in soil biochemical properties which are affected by ecological stress from natural phenomena or anthropogenic activities (Abd El-Azeem et al. 2013). Soil enzymes help in the biochemical transformations of pollutants in the soil and also function as a measure of soil fertility (Chinyere et al. 2013). Heavy metals cause long-term hazardous effects on soil ecosystems and have an inhibitory influence on soil enzymes (Chen et al. 2005; Khan et al. 2007).

The objectives of this study were (1) to determine the content of selected heavy metal forms (total and available forms of Zn, Cu, Pb and Ni) in soil under illegal dumping urban sites; (2) the environmental pollution of soil by potential pathogen microorganisms and (3) the impact of anthropogenic sources on the activity of selected enzymes (dehydrogenases, catalase, alkaline and acid phosphatase) in soil surface horizons.

Materials and methods

Location of soil sampling

Soil samples were collected in autumn (September) 2014 out of three illegal landfills located in the forestall area located in the district of Łęgowo, in the southeastern part of Bydgoszcz (53°12'N, 18°01'E; the Kujawy and Pomerania Province, central Poland). For this region the annual mean temperature is 8.9 °C and the mean annual precipitation is 521 mm. In September 2014 the precipitation did not exceed 523 mm, with an average temperature 11.0 °C and prevailing winds from the western direction. They were the landfill spots encompassing the surface of 20–35 m² of different morphological composition (Table 1). They were marked as A, B, C. All of them were located on sandy soils. Those soils were classified as Brunic Arenosols (IUSS WRB 2014). The control point was located away from the designated landfill (approx. 200 m—in forest soils in the Bydgoszcz Forest). The area of the Bydgoszcz Forest is covered by the habitats of fresh forest (Bs'w) where the share of coniferous species

Table 1 Morphological composition of the dumping waste sites

Objects	Morphological composition
A	Ceramic construction materials, rubble, roofing paper, remnants of mortar, foamed concrete, foamed polystyrene
B	Car tires, plastic containers from food products, plastic foil, metal elements, metallic foils, paper, metal containers of paint, glass bottles, elements of light bulbs and fluorescent tubes, medical packing and wrappings
C	The waste of organic origin from households and cut grass as well as residue from garden maintenance

in the tree stand reaches beyond). After the waste removal, soil sample was taken with the Egner stick from the depths of 0–25 cm. The samples were taken from the same depth of the Control point. A total of 36 samples were analyzed. Each soil sample was a mixture (Control—five sub-samples and A, B, C after ten sub-samples) taken randomly in the selected area.

Soil analysis

Physicochemical parameters of the soil

In the air-dried soil samples with disturbed structure, sieved through ϕ 2 mm, some physicochemical properties were determined according to commonly used procedures in soil science laboratories: the granulometric composition with the laser diffraction method applying the Mastersizer MS 2000 analyser, pH w H₂O and pH in 1 M KCl measured potentiometrically (PN-ISO 10390 1997), organic carbon (C_{org}) with the Tiurin method by sample oxidation in the mixture of K₂Cr₂O₇ and H₂SO₄ (PN-ISO14235 2003), the total content of zinc, copper, lead and nickel after the mineralization in the mixture of HF + HClO₄ acids with the Crock and Severson's method (1980) and its easily available forms, extracted with DTPA (1 M diethylenetriaminepentaacetic acid), according to Lindsay and Norvell (1978). The total contents and mobile forms were determined using the method of atomic absorption spectroscopy with the PU 9100X spectrometer (Philips).

Microbial analysis

The soil samples, transported to the laboratory in sterile plastic bags, were stored at 4 °C about 18 h for microbial analysis. One-gram soil samples diluted in 9 ml 0.9% NaCl (10^{-2} – 10^{-5}) were analyzed. Quantitative analyses were carried out based on the calculation of the most probable number of microorganisms (MPN method).

For isolation of *Salmonella* spp., two media were used. In the first stage, soil samples were blended in 1% peptonic water (Merck, 7228) and incubated at 37 °C for 24 h. Then, 0.1 ml of the material was transferred to selectively multiplying liquid medium following Rappaport (Merck, 10236) and incubated at 43 °C for 24 and 48 h. Next, the material was sieved to selective agar medium BPLA, following Kaufmann (Merck, 7236), and incubated at 37 °C for 24 h (Atlas 2010). Final identification involved serological tests (polyvalent serum HM).

Quantitative determination of *Escherichia coli* The material samples were added to liquid MacConkey medium and incubated at 43 °C for 24 h. The material was then sieved into agar with tergitol and TTC (Merck, 7680) and incubated at 43 °C for 24 h. Final identification involved biochemical tests API 20E (Biomérieux, 20100/20160).

Other coliform bacteria were isolated on Endo agar and incubated at 37 °C for 24 h (Atlas 2010).

Quantitative determination of enterococci Broth bouillon with glucose and azide was used as an enriching medium for selective growth of *Enterococcus* spp. (Merck, 1590). After 48 h of incubation in 37 °C, the material was streaked to agar with esculin and azide (Merck, 5222). The serological test for confirmation of presumption colony was carried out (Phadabac Strep D Test, Karo Boule Diagnostics AB, Huddinge, Sweden).

Biochemical measurements

Field-moist samples were sieved (2 mm mesh) and stored in a plastic box at 4 °C for not less than 2 days in order to stabilize the microbial activity and then were analyzed for catalase, dehydrogenases and phosphomonoesterases activity within one week.

The activity of selected redox enzymes: namely dehydrogenases activity (DEH) with 2, 3, 5-triphenyl-tetrazolium chloride and a measurement of triphenylformazan (TPF) for absorbance at 546 nm was determined and was expressed as mg of TPF kg⁻¹ soil 24 h⁻¹ according to Thalmann method (1968). The activity of catalase (CAT) [E.C. 1.11.1.6] in soil with the Johnson and Temple method (1964) with 0.3% hydrogen peroxide solution as a substrate was determined. The residual H₂O₂ was determined by titration with 0.02 M KMnO₄ under acidic conditions.

The activity of selected enzymes were also determined, representing the class of hydrolases: alkaline phosphatase [E.C. 3.1.3.1] (ALP) and acid phosphatase [E.C. 3.1.3.2] (AcP), with the method of Tabatabai and Bremner (1969). It is based on the colorimetric assaying of released substrate: p-nitrophenylphosphate (pNP) after the incubation of soil with of MUB (Modified Universal Buffer) at pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase samples for 1 h at the temperature of 37 °C.

Statistical analysis

With the values of the contents of heavy metals recorded in the soil, geochemical parameters were calculated evaluating the anthropogenic effect on the soil environment.

Contamination Factor (CF):

$$CF = C_{0-1}/C_n$$

where C₀₋₁—the mean content of metals from at least five sampling sites, C_n—geochemical background.

The sum of CF for all studied metals yields the so-called contamination degree (C_{deg}) of the ecosystem.

In the paper for background geochemical the following values for Zn—18.0 mg kg⁻¹, Cu—4.0 mg kg⁻¹, Pb—7.7 mg kg⁻¹, Ni—4.0 mg kg⁻¹ (Czarnowska 1996).

Table 2 Selected physicochemical properties

Objects	pH H ₂ O		pH KCl		Organic-C g kg ⁻¹		Particle size fractions %					
							Sand		Silt		Clay	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Control	4.2	4.75	3.85	4.39	9.5	14.2	79.89	90.62	8.57	17.9	0.81	2.21
A	6.74	8.56	6.54	8.02	2.3	4.10	80.5	90.25	8.81	18.65	0.85	1.71
B	4.42	4.83	4.35	4.67	1.60	4.80	77.55	93.1	4.93	20.54	0.85	1.97
C	6.98	7.34	6.85	7.30	10.8	26.5	79.98	91.25	7.37	17.67	0.98	2.35

A, B, C described in Table 1

The availability factor (AF) as suggested by Obrador et al. (2007) was applied for this purpose. It is expressed as follows: $AF = \frac{DTPA_{Cu,Zn,Pb,Ni}}{Total_{Cu,Zn,Pb,Ni}} \times 100$, where AF—availability factor (%), $DTPA_{Cu,Zn,Fe,Mn}$ —amounts of DTPA-extractable micronutrients (mg kg⁻¹), Total Cu, Zn, Fe, Mn—total extractable micronutrients contents (mg kg⁻¹).

The % inhibition of the activity of the tested enzymes was calculated based on the following formula: $I_N = [(A/A_c) - 1] \times 100$, (Kucharski et al. 2009), where: I_N [%] activity inhibition, A—enzyme activity in contaminated soil, A_c —enzyme activity in control soil.

All the assays were made in three reps; the paper demonstrates the arithmetic means of the results. The classical statistical parameters such as the maximum and minimum, mean, standard deviation (SD), coefficient of variation (CV%) were evaluated using STATISTICA version 9.0 Software.

The coefficient of variation of the parameters analyzed was calculated as follows:

$$CV = (SD/X) \times 100$$

where CV [%]—coefficient of variation (%), SD—standard deviation, X—arithmetic mean. Coefficient of variation (CV) was used to reflect the degree of discrete distribution of different heavy metals contents and activity of selected enzymes and to indicate indirectly the activity of the selected elements in the examined environment. Values where 0–15, 16–35, and >36% indicate low, moderate or high variability, respectively. Normality of the data was determined using the Shapiro–Wilk test. The relations between the studied properties were estimated using a correlation analysis based on Pearson's correlation coefficients ($P < 0.05$).

Principal components analysis (PCA) was performed using a correlation matrix for heavy metals, physicochemical properties and soil enzyme activities. The first two principal components (PC1 and PC2) were selected for further interpretation of the results.

Results and discussion

Physical and chemical characteristics of soils

Basic physicochemical properties of the soil samples are presented in Table 2. The dominant fraction in the soil samples studied was the sand fraction from 2.0 to 0.05 mm in diameter (77.55–93.1%). The content of clay fraction (particle size < 0.002 mm) was low and ranged from 0.81 to 2.35% (Table 2). The samples were classified only as two grain size groups: sand and loamy sand (according to USDA). According to the literature (Malinowski et al. 2012), the majority of illegal dump wastes are found in Poland in light soils with the granulometric size of loose sand, which contribute to the change of their properties, especially the chemical ones. Marking of the exchangeable and hydrolytic acidity allowed to determine the analyzed soil, which is in the acidic to alkaline range. Values expressed in H₂O pH fluctuated in the range of 4.2–8.56 while in 1 mol KCl from 3.85 to 8.02. The highest pH was recorded in the case of landfill B where building and renovating waste was stored. Rubble waste with calcic pointing contributed to the reduction of soil acidification on which they were stored. Such materials do not cover the soil evenly. Therefore, in sandy soils with strong water permeability the soil acidity is reduced on a point basis within the landfills (Bielinska and Mocek-Plóćiniak 2009). The control point and all the illegal landfill sites were located in the coniferous forest-soils area. Acidic soils are common in forest ecosystems, and the pH is the element influencing the transformation of organic matter in the soil (Tonon et al. 2010). The content of organic carbon in the soil developed in a wide range of 1.6–26.5 g kg⁻¹ (depending on the object). The soil samples collected from the object Control contained the organic carbon in the range of 9.5–14.25 g kg⁻¹. The main source was the gradual accumulation of material derived from the fallen leaves of trees and dead wood undergrowth (Brogowski and Chojnacki 2013). The maximum amount of this parameter was

Table 3 Total content of heavy metals in the soil samples

Parameters	Samples (<i>n</i>)	Min (mg kg ⁻¹)	Max (mg kg ⁻¹)	Mean (mg kg ⁻¹)	SD	CV %
Zn						
Control	6	13.2	17.83	15.37	2.48	16.11
A	10	16.8	25.5	20.93	4.39	20.96
B	10	22.83	45.94	34.19	10.02	29.31
C	10	54.83	276	165.83	127.09	76.64
Cu						
Control	6	3.05	5.53	4.06	1.12	27.66
A	10	2.30	5.68	4.11	1.51	36.60
B	10	4.53	9.58	6.82	2.15	31.67
C	10	18.55	108	47.84	41.80	87.39
Pb						
Control	6	4.91	7.62	6.13	1.40	22.82
A	10	0.20	4.12	2.02	1.98	97.81
B	10	4.31	9.24	5.82	2.30	39.53
C	10	4.84	21.48	15.80	7.83	49.54
Ni						
Control	6	2.50	4.65	3.68	0.92	24.91
A	10	3.50	4.18	4.03	0.42	10.42
B	10	4.98	6.22	5.55	0.53	9.56
C	10	7.03	14.25	12.80	3.99	31.25

A, B, C described in Table 1

observed in samples taken directly from the landfill C (26.5 g kg⁻¹), a part of which consisted of most organic waste. Lower organic carbon content was observed in the case of landfills A and B. Due to the deposited waste, no fresh organic matter was provided, and as a result of the mineralization, water-soluble humus (fulvic acids) could be washed out into the soil and accumulated in the lower layer of bedrock. The content of organic carbon is one of the most essential soil parameters, and so its deficit affects other physicochemical and biological soil parameters.

Content of the selected heavy metals in soil

The total amount of contamination derived from the landfills depends on the type of the deposited waste. Nabulo et al. (2008) found that the accumulation of heavy metals in surface soil level is closely correlated with the operation of the illegal deposit of waste. The high content of zinc, copper, lead and nickel was found directly beneath the waste in samples taken from the object C (Table 3). Little uniformity of the results was stated, which high CV coefficients confirmed, amounting to 76.64% for zinc, copper, 87.39, 49.54% lead and 31.25% for nickel. The contents were significantly higher than the concentration of these metals in samples taken from the Control point and landfill A. The contents were significantly higher than the concentration of these metals in samples taken from the Control point and object A. The highest concentration from

analyzed heavy metals characterized zinc and lead, and the lowermost copper. The high concentration of heavy metals was stated in the surface soil horizons (0–25 cm), which was mainly of anthropogenic origin. It was also confirmed by the studies of Dusza et al. (2013) and Nabulo et al. (2008). The elevated contents of heavy metals in the surface horizons of sandy soils are usually a result of an increased organic matter and the soil capacity for retaining heavy metals. According to Niedźwiecki et al. (2007), uncontrolled waste landfills located in sandy areas may contaminate the soil's surface layers with heavy metals, particularly copper and zinc. However, the quoted authors, as well as Szymańska-Pulikowska (2003), maintain that municipal waste is characterized by a very varied content of heavy metals, and the intensity of environmental changes occurring under their influence is connected with the quality of the waste, frequency and time of storage and supply of the dump with illegal domestic sewage discharge, particularly on uncontrolled dumps.

A similar relationship was observed in the case of the contents of available forms for plants. The highest concentration of bioavailable forms zinc, copper and lead were also found in soil samples taken from C (Table 4). The contents were significantly higher than the concentration of these metals in samples taken from the control point and A. The mobility of heavy metals in the storage determines the environmental conditions, the water content and the chemical bonding with the largest weight to the fine-

Table 4 DTPA-extractable forms of heavy metals in the soil samples

Parameters	Samples (<i>n</i>)	Min (mg kg ⁻¹)	Max (mg kg ⁻¹)	Mean (mg kg ⁻¹)	SD	CV %
Zn						
Control	6	2.07	2.13	2.10	0.03	1.69
A	10	1.13	2.70	1.9	0.87	46.03
B	10	9.99	21.82	15.82	6.28	39.71
C	10	6.78	100.5	53.96	54.4	100.9
Cu						
Control	6	0.35	0.40	0.38	0.02	6.55
A	10	0.35	0.98	0.58	0.30	51.33
B	10	3.71	7.00	5.32	1.72	32.46
C	10	2.03	10.71	6.37	5.01	78.71
Pb						
Control	6	0.36	1.97	0.87	0.75	85.75
A	10	0.36	1.99	1.08	0.73	67.59
B	10	0.39	2.84	1.42	1.11	77.96
C	10	2.03	10.71	5.72	4.13	72.25
Ni						
Control	6	0.13	1.98	0.79	0.84	107.2
A	10	0.21	1.13	0.94	0.88	93.34
B	10	1.34	28.45	12.6	12.8	102.0
C	10	0.19	7.49	2.68	3.40	126.9

A, B, C described in Table 1

Table 5 Coefficients and degrees of soil contamination

Variable	CF				CF in C_{deg} (%)				AF (%)			
	Control	A	B	C	Control	A	B	C	Control	A	B	C
Zn	0.85	1.50	2.44	11.85	24.39	39.43	38.80	27.67	13.66	9.08	46.27	32.54
Cu	1.02	1.03	1.71	11.96	29.12	27.11	27.12	27.94	9.35	14.11	78.00	13.31
Pb	0.80	0.26	0.76	15.80	22.84	6.91	12.02	36.91	14.19	54.46	24.45	36.20
Ni	0.92	1.01	1.39	3.20	26.40	26.56	22.06	7.48	21.47	23.32	22.91	20.94
C_{deg}	3.49	3.79	6.29	42.81								

CF contamination factor, CF in C_{deg} degree of contamination, AF availability factor

grained material, the precipitation of the salt, the precipitation of iron minerals or phosphorus and binding with the organic phase of the soil. The critical phase for the launch of metals is the acidic phase. The increase in the redox potential and pH decline, which occurs in the acidic waste storage phase with a small amount of organic matter, leads to increased mobility of metals such as lead, cadmium, zinc, copper and mercury (Tałała 2014; Liu and Sang 2010). Such conditions could occur in B landfills where in the low pH content of available forms of analyzed heavy metals was lower than in C. However, in the case of zinc and copper availability factor (AF) was the highest and reached 46.27% for zinc and 78% for copper (Table 5). In their studies on the availability of selected heavy metals in soils, Sidhu and Sharma (2010) have reported that the total

content of these micronutrients increased with an increase in clay and silt, whereas the DTPA-extractable levels decreased with increasing pH and calcium carbonate content. Similar data have been reported by Mathur et al. (2006) who showed that $DTPA_{Zn}$ correlated among others significantly and negatively with pH, but positively with organic carbon. In the analyzed soil samples, no correlation between soil pH and the content of the available elements was found. Increasing the organic matter in the case of object C contributed to the rise of heavy metal content of both forms. Land rich in organic matter actively retains metallic elements. The metal concentration depends on the possibilities of creating organic and chelate soil compounds. Durability of chelates depends on the pH of the soil and the type of metal ion. Creating organometallic

Table 6 Occurrence of potential pathogenic bacteria in the soil under illegal dumping sites

Objects	<i>Escherichia coli</i> (MPN g ⁻¹)	Total coliforms (MPN g ⁻¹)	<i>Salmonella</i> spp. (MPN g ⁻¹)	<i>Enterococcus</i> spp. (MPN g ⁻¹)
Control	n.d*	1600.0	n.d	250.0
A	n.d	1600.0	n.d	n.d
B	9.5	1750.0	n.d	n.d
C	96.0	3000.0	25.0	45.0

*n.d. not detected

complexes in the soil is very important in order to prevent the toxic metal ions leaching the soil, and it also detoxifies and reduces uptake of the toxins by plants (Gustafsson et al. 2003). To confirm such interdependencies, a correlation analysis was performed, which showed a significant positive correlation between the content of organic carbon and the content of total amount of copper ($r = 0.573$, $P < 0.05$), lead ($r = 0.615$, $P < 0.05$) and nickel ($r = 0.705$, $P < 0.05$; Table 9). In the publication, there is a lot of information stating that the solubility of metal sorbent depends primarily on the soil, pH, the concentration of the inorganic ligands and soluble humic acids in soil solution. The solubility of the metals depends mainly on the metal loading over soil sorbents, pH, and the concentration of inorganic ligands and dissolved organic matter (DOM) in the soil solution (Krosshavn et al. 1993; Dube et al. 2001; Weng et al. 2002; Ashworth and Alloway 2008).

The contamination coefficient (CF) allowed for the classification of the examined soils to the appropriate group, depending on the geochemical background factor. Contamination factors for four analyzed heavy metals in all sites are summarized in Table 5. According to the criterion developed by Håkanson (1980), the analyzed soil from point Control, object A and B was characterized by a low coefficient of pollution for heavy metals. However, samples taken from the C landfill have shown moderate contamination in nickel and significantly high contamination in other elements (Table 5). Similarly, Ideriah et al. (2010) reported that the waste dumps contribute to high copper levels in the soils. The overall assessment of the soil pollution state was tested based on the degree of contamination (C_{deg}). The degree of contamination ranged from 3.49 to 42.81, which according to the Håkanson classification (1980), provides a low degree of soil pollution with heavy metals in positions Control, A and B and very high in the C position (Table 5). The overall pollution degrees of analyzed heavy metals were in the order of $Zn > Cu > Pb > Ni$.

Microbiological parameters

Microbiological research has shown that there were potentially pathogenic fecal bacteria in the examined soil.

The bacilli of *E. coli* were detected under two landfills B and C, but far more were isolated where organic waste was present in C landfill (96 MPN g⁻¹) (Table 6). *Salmonella* bacteria occurred only in the soil at C landfill at 25 MPN g⁻¹. According to Holley et al. (2006), bacilli of *Salmonella* spp. survival in soil range from 6 to several hundred days, which create a real risk for soil contamination and for the growing plants. Other coliform bacilli were detected on all of the test facilities at 10³ level. Organic waste of food scraps and lawn clippings were in particular the source of pathogenic microorganisms proliferation including *Escherichia coli*, fecal coliforms, *Salmonella* spp. or fecal streptococci. In addition, fecal bacteria may be present in the feces of wild animals and especially in the case of organisms with gastrointestinal tract disease. Animals living in the forest and the birds seeking food waste on landfills may be the source of bacteria transmission. Such situation probably occurred in the case of fecal streptococci, most of which was obtained in the control point (250 MPN g⁻¹) (Table 6). The feces of wild animals in forest areas can lead to spot contamination of soil and undergrowth. Additionally, in favorable conditions, bacteria can penetrate deep into the soil and into the groundwater leading to the source of potential danger (Edrington et al. 2009; Grisey et al. 2010).

According to You et al. (2006), the environment of the soil does not create optimum conditions for existence of the fecal bacteria. The research performed by Yamahara et al. (2012) shows that some of the fecal bacteria multiply inside the soil and adsorb on the particle of the soil or move deeper into the soil. The study shows that the most favorable conditions for the development of potentially pathogenic *E. coli* bacteria, *Salmonella* spp. and fecal streptococci prevailed on the C landfill. However, their presence was not identified in soil samples collected from the landfill A. The waste, rich in organic matter, accumulated on the C most likely contributed to their propagation in a landfill compared with A and B which are characterized by a completely different morphology. It is important to stress the fact that the possibility of survival of the bacteria of enteric origin (fecal) in the soil depends on many determinants including the temperature, pH, moisture, soil type and organic carbon content (Gallagher et al.

2012; Yamahara et al. 2012). The presented results of the statistical analysis revealed a positive correlation between the content of organic carbon and total coliforms ($r = 0.709$, $P < 0.05$) and *E. coli* ($r = 0.690$, $P < 0.05$). Additionally, high correlation of coefficient was obtained between the bacteria and the selected heavy metals, e.g., *E. coli* and total nickel content ($r = 0.936$, $P < 0.05$), total lead ($r = 0.819$, $P < 0.05$) (Table 9). The results show that the most dangerous bacteria exist in the soil under the C landfill and this is where the greatest risk of soil contamination exists. Increasing the amount of organic matter in the soil under C helps to minimize the absorption of heavy metals by microorganisms. The formation of metal–organic complexes in soil is very important due to the prevention of leaching of toxic ions of heavy metals from soil, as well as their partial detoxication and limiting the uptake by microorganisms (Gustafsson et al. 2003; Khan et al. 2010).

Enzyme activities

All of the enzyme activities (dehydrogenases, catalase, alkaline and acid phosphatase) showed fluctuating results in the present study (Table 7). The highest activity of the enzymes in the soil was obtained from C (mean DEH 5.202 mg TPF kg⁻¹ soil 24 h⁻¹, CAT 66.00 mM H₂O₂ - kg⁻¹ soil min⁻¹, AIP 1.657 mM pNP kg⁻¹ soil h⁻¹, AcP 2.139 mM pNP kg⁻¹ soil h⁻¹). This was due to high

organic carbon content, which was confirmed by the positive values of the correlation coefficient between the content of total organic carbon and dehydrogenases ($r = 0.524$, $P < 0.05$), catalase ($r = 0.627$, $P < 0.05$) and alkaline phosphatase ($r = 0.734$, $P < 0.05$) (Table 9). Mineralization of deposited waste has started on the area which is covered by soil (C), plants, leaves of the trees, waste of organic origin from households and cut grass as well as residue from garden maintenance. Easily decomposable organic materials are in advanced stage of decomposition. According to Lemanowicz and Krzyżaniak (2015), organic carbon served as a precursor for enzyme synthesis and played a vital role in the physical stabilization of the enzyme. Soil organic matter is an organic carrier of soil enzymes (Wang et al. 2012). The enzyme activity of the controlled soil was higher from the soil from A and B point, but lower than C. According to Halasz et al. (2011), high enzymes activity of the control area can be explained by the absence of wastes and the domination of plants. We found no significant correlation between enzyme activities and soil pH. Catalase is active over a wide pH range, and its activity does not drop until the pH is below 3.5. Phosphatases have been often closely correlated with soil pH. A higher activity of acid phosphatase comes from the fact that phosphomonoesterases are the enzymes most susceptible to the changes in the soil reaction; the optimum pH of soil for the activity of alkaline phosphatase is 9.0–11.0, and for

Table 7 Statistical data of soil enzymes activity

Parameters	Samples (n)	Min	Max	Mean	SD	CV %
DEH						
Control	6	0.216	0.480	0.330	0.124	37.70
A	10	0.096	0.144	0.120	0.019	16.32
B	10	0.072	0.192	0.126	0.063	50.09
C	10	2.136	8.304	5.202	3.540	68.06
CAT						
Control	6	10.64	18.96	15.15	4.389	28.97
A	10	6.285	15.85	11.82	4.754	40.22
B	10	8.285	11.14	9.661	1.273	13.18
C	10	40.85	91.41	66.00	27.28	41.33
AIP						
Control	6	1.030	1.188	1.109	0.081	7.367
A	10	0.548	0.859	0.693	0.161	23.28
B	10	0.234	0.559	0.389	0.177	45.68
C	10	1.353	1.990	1.657	0.340	20.51
AcP						
Control	6	1.508	2.270	1.896	0.409	21.60
A	10	0.408	1.065	0.723	0.337	46.71
B	10	0.442	2.372	1.400	1.067	76.23
C	10	1.771	2.552	2.139	0.412	19.25

DEH dehydrogenases (in mg TPF kg⁻¹ soil 24 h⁻¹), CAT catalase (in mM H₂O₂ kg⁻¹ soil min⁻¹), AIP alkaline phosphatase (in mM pNP kg⁻¹ soil h⁻¹), AcP acid phosphatase (in mM pNP kg⁻¹ soil h⁻¹)

Table 8 Inhibition index (I_N) to contamination with illegal dumping

Objects	DEH	CAT	AIP	AcP
A	−63.6	−21.9	−37.5	−61.9
B	−61.8	−36.2	−64.9	−26.2
C	1475	335	49.5	12.8

A, B, C described in Table 1

acid phosphatase—4.0–6.5 (Wittmann et al. 2004). The enzyme activities (dehydrogenases, catalase and alkaline phosphatase) were correlated with organic carbon, an effect that was not observed for acid phosphatase activity (Table 9). Higher organic matter provides a better environment for stabilizing and protecting extra cellular enzymes. Moreover, higher C levels support greater microbial biomass and thus have more enzymatic activity (Balota et al. 2011). There was no negative effect of heavy metals on the activity of the enzymes. This was because of the content of metals in the soil. At low concentrations, metals can stimulate the activity as well as many enzymes contain metal ions (Zn, Fe, Ca, Mg, Cu, Mn), the presence of which is necessary for their catalytic activity. This was confirmed by obtaining a positive significant correlation coefficient between the content of total lead and dehydrogenase activity ($r = 0.891$, $P < 0.05$), catalase ($r = 0.892$, $P < 0.05$), alkaline phosphatase ($r = 0.762$, $P < 0.05$) and

acid phosphatase ($r = 0.627$, $P < 0.05$) (Table 9). The activity of the enzymes in the soil from the illegal landfills of A and B underwent inhibition (A: $DEH < AcP < AIP < CAT$ and B: $AIP < DEH < CAT < AcP$) (Table 8). However, the soil sampled from illegally deposited C landfill caused an increase in the activity of both enzymes and hydrolytic oxidation–reduction. Dehydrogenase proved to be the enzyme most sensitive to changes in the soil environment as evidenced by the value of inhibition index (IN) (A = −63.6, B = 61.8 and C = 1475). Dehydrogenases is the most important soil enzyme and a frequently used test for determining the influence of the various pollutants (heavy metals, pesticide, crude oil, etc.) on the microbiological quality of soil (Margesin et al. 2000). Their activity can be inhibited from 10 to 90% depending on the soil contamination.

Table 10 displays the factor loadings, as well as the eigenvalues. Three principal components were extracted from the available dataset that explained a total variance of approximately 77% (Table 10). The number of significant principal components is selected on the basis of the Kaiser (1960) criterion with eigenvalue higher than 1. Factor 1 (PC1) is responsible for 55.34% of the total element variables and indicated great correlation with total and available heavy metals (except Ni_{DTPA}), the activity enzymes, number of *Escherichia coli* and total coliforms. The higher loadings of total and available heavy metals increased

Table 9 Pearson's correlation coefficients

Variables		Equation	r	r^2
Dependent	Independent			
Total copper	Total organic carbon	$y = 7.0314 + 0.2073x$	0.537	0.328
Total lead	Total organic carbon	$y = 3.4495 + 0.9184x$	0.615	0.378
Total nickel	Total organic carbon	$y = -0.2062 + 1.6107x$	0.705	0.497
Dehydrogenases	Available zinc	$y = -0.0166 + 0.0792x$	0.949	0.902
Dehydrogenases	Total organic carbon	$y = -0.0827 + 0.1484x$	0.524	0.275
Catalase	Total organic carbon	$y = 7.5574 + 1.7595x$	0.627	0.393
Alkaline phosphatase	Total organic carbon	$y = 0.5524 + 0.0398x$	0.734	0.538
Dehydrogenases	Total lead	$y = -1.361 + 0.3768x$	0.891	0.793
Catalase	Total lead	$y = -2.1856 + 3.739x$	0.892	0.796
Alkaline phosphatase	Total lead	$y = 0.5022 + 0.0618x$	0.762	0.581
Acid phosphatase	Total lead	$y = 1.0221 + 0.0696x$	0.568	0.323
Dehydrogenases	Total zinc	$y = -0.4209 + 0.0316x$	0.984	0.973
Catalase	Total zinc	$y = 7.8495 + 0.3014x$	0.950	0.903
Alkaline phosphatase	Total zinc	$y = 0.6927 + 0.0046x$	0.742	0.552
Total coliforms	Total organic carbon	$y = 57.790x + 1392.9$	0.709	0.502
<i>E. coli</i>	Total organic carbon	$y = 2.355x - 4.0452$	0.690	0.475
<i>E. coli</i>	Total nickel	$y = 7.302x - 27.386$	0.936	0.876
<i>E. coli</i>	Total lead	$y = 4.176x - 10.907$	0.819	0.670
Total coliforms	Total nickel	$y = 143.3x + 1053.2$	0.770	0.593
Total coliforms	Total lead	$y = 91.43x + 1306.7$	0.750	0.563
<i>Salmonella</i> spp.	Total nickel	$y = 2.0181x - 6.961$	0.705	0.496

Table 10 Values of the three extracted factor loadings for 22 elements

No and elements	Component matrix		
	PC1	PC2	PC3
1. Zn _{DTPA}	−0.888	−0.106	−0.046
2. Cu _{DTPA}	−0.756	−0.153	−0.054
3. Pb _{DTPA}	−0.924	−0.017	−0.052
4. Ni _{DTPA}	−0.626	−0.182	−0.279
5. Zn _{Tot}	−0.938	−0.029	−0.040
6. Cu _{Tot}	−0.909	−0.137	−0.222
7. Pb _{Tot}	−0.929	−0.161	0.156
8. Ni _{Tot}	−0.899	0.145	0.027
9. Corg	−0.667	−0.085	0.154
10. pH H ₂ O	−0.317	0.768	−0.418
11. pH KCl	−0.362	0.791	−0.392
12. Sand	0.095	0.411	0.807
13. Silt	−0.049	−0.376	−0.805
14. Clay	−0.462	−0.492	−0.385
15. Dehydrogenases	−0.962	−0.002	0.021
16. Catalase	−0.974	0.001	0.050
17. Alkaline phosphatase	−0.816	−0.113	0.223
18. Acid phosphatase	−0.562	−0.543	0.276
19. <i>Escherichia coli</i>	−0.836	0.262	0.231
20. Total coliforms	−0.768	0.179	0.133
21. <i>Salmonella</i>	−0.663	0.379	0.344
22. <i>Enterococcus</i> spp.	0.174	−0.581	0.508
Variation (%)	52.34	12.7	11.55
Eigenvalue	11.51	2.80	2.54

Bold values are statistically significant

concentration probability of anthropogenic sources (illegal dumping sites). Factor 2 (PC2) is dominated by pH H₂O and pH KCl, accounting for 12.75% of the total variance, which may suggest that the two elements have different sources. Factor 3 (PC3) is dominated by sand and silt and accounts for 11.55% of total variance (Fig. 1). The distribution of these elements is mainly controlled by natural parent materials. According to Gergen and Harmanescu (2012), a positive score means that the concentration of variables increases along with the PC axis; a negative score means that the concentration of variables decreases along the axis, and a score near 0 means that the concentration is poorly (linearly) related to the PC axis.

Conclusion

Information about the state and the direction of changes happening in the soil environment in the uncontrolled landfills is delivered by marked biological and physicochemical

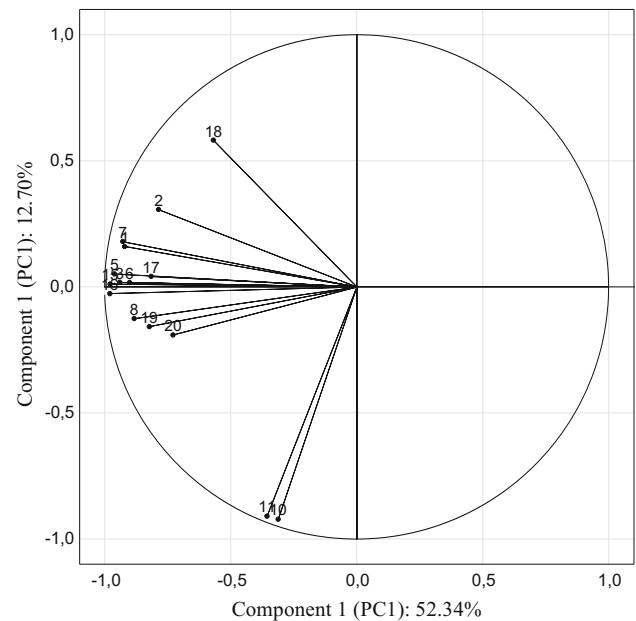


Fig. 1 Configuration of variables in the system of the first two axes of principal components PC1 and PC2 of the content heavy metals, enzyme activities, counts of bacteria and physicochemical properties in soil under illegal dumping sites (numeric references are found in Table 10)

soil parameters (microbiological and biochemical). In the tested samples, it was found that depending on the waste composition, the physicochemical properties of soil changed. Uncontrolled dumps significantly affect the increase or decrease in the pH of the tested soil and organic matter content.

The waste stored at the landfills affected the contamination of the soil by heavy metals to varying degrees. In the study, it was found that the greatest risk of soil contamination by pathogenic bacteria occurred in the soil under the landfill of organic waste.

The study concluded that the composition of illegal A and B dumping sites had a more negative impact on the soils than C (accumulated wastes of organic origin) with the enzymes. The negative inhibition index (IN) for selected enzymes was found in the soil from the area of A and B dumping sites; the highest IN was determined in C soils. Our results have shown that the addition of organic residues to illegal dumping sites (C) has a variety of effects on enzyme activities and organic carbon content.

The results of the study show that it is necessary to continue research on the heavy metals content and biological properties of the soil affected by illegal dumping sites. However, heavy metals and other potential pollutants contribute to environmental pollution in the municipal solids.

As confirmed by studies, illegally operated waste depots are a threat to the natural environment. The work in this

field should be continued because it will help assess the environmental consequences of the gradual accumulation of contaminants in the soils surrounding the unlisted landfills.

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